NOTES ON THE ACTIVITY OF EARTHWORMS

VI. Periodicity in the oxygen consumption and the uptake of feed

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Introduction

Periodicities in biological processes have been mentioned by many authors; mostly they are daily or 'solar' rhythms. Ralph (1957), however, deduced from his experiments that earthworms do not only show a 'solar', but also a 'lunar' rhythm in their activity.

Cole (1957) described a combined 'solar' and 'lunar' rhythm in the activity of the unicorn, which of course does not imply that earthworms should not show such a rhythm, but his results urge the utmost care in arranging figures.

A most interesting observation of Ralph (*l.c.*) is the dissimilarity of the curves representing oxygen consumption and motor activity, suggesting a partly suspended oxygen uptake during periods of greater activity.

If this should be an inherent quality in earthworms, it would ecologically be a most useful adaptation. It will enable the animals to burrow into deeper soil layers which may be poor in oxygen, provided that after some time they can withdraw to layers with more oxygen. Here they can rest and restore their oxygen reserve by resynthesizing glycogen from the lactic acid accumulated during their stay under poor aerobic conditions (*cf.* Davis and Slater, 1928).

Methods

One of the disadvantages of working with earthworms in research on biological rhythms is that the different aspects of activity have to be studied on different groups of animals. Ralph (1957) studied oxygen consumption and locomotion; in the studies, reported here, oxygen consumption and feed uptake of different groups of worms were considered as two aspects of earthworm activity. So far no satisfactory apparatus has been designed in which different activities of the same animals can be studied simultaneously, though technical experiments in this field are well underway.

Oxygen uptake was studied in an apparatus of the type described by Doeksen (1957). In the present experiment all glass parts were replaced by a block of Perspex (Plexiglas), making the apparatus more compact and manageable (Fig. 1). The respiration chamber was divided by perforated perspex into three horizontal compartments, in each of which one *Lumbricus terrestris* was kept. This enabled a number of worms to be used at the same time, but prevented them from rolling up into a knot, which might influence their activity. The bottom of the compartments was covered for

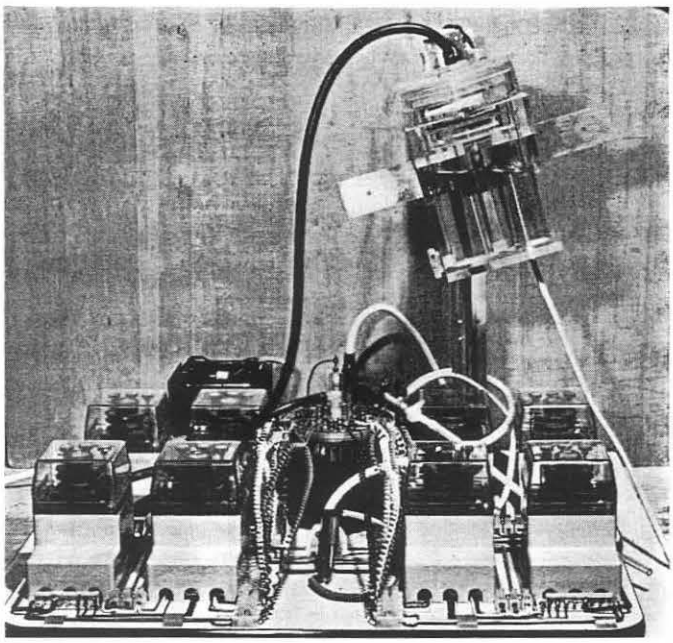

FIG. 1. Respiration apparatus used in the experiment. Top-right: respiration chamber and CO_2-absorption vessels, tilting slowly from one side to the other; lower-centre: vessel with $CuSO_4$, 1 central anode and 24 kathodes; lower-left and right: 8 time switches with 3 day-programmes each.

2/3 with wet nylon cloth, providing enough moisture for the worms to stay in good condition, but not a substrate for micro-organisms which could otherwise interfere with oxygen consumption.

The vacuum in the respiration chamber, resulting from CO_2-adsorption by NaOH, is made up by oxygen produced electrolytically at a platinum anode from a solution of copper sulphate. Cu is precipitated on the kathodes of stainless steel; 24 kathodes were used, which were switched on consecutively, one for each hour of the day. The worms were changed every 49 hours and the experiment lasted for 49 days. Disturbance of the worms brought about by their replacement thus was regularly distributed over the day and there were no peaks in oxygen consumption caused by these disturbances.

The worms used in the experiments had been in captivity for at least half a year. Before they were used in the experiment, they were kept in wet cellulose powder (220% of water) for two days and afterwards for 2–3 days in boxes with soaking wet pieces of foam plastic of *c.* 1 × 1 × 1 cm, after which treatment their intestines were practically empty. The disturbances caused by these preparations were always at the same time of the day, *viz.* at 14.30 hr.

Before and during the experiments, the animals were kept at a constant temperature of 8 °C and a constant diffuse illumination.

At the end of the experiment the kathodes were rinsed, dried at 105 °C and weighed. 1 mg of Cu corresponds with 176 µl of O_2.

The fresh weight and dry weight of the worms (dried in vacuo at 50 °C) were determined.

To determine the periodicity in feed uptake, the worms to be used were kept for

4–5 days in wet cellulose powder (220% of water), refreshed after 1 and 3 days, to make their intestines free of soil. Eight specimens of *L. terrestris* were kept in 160 g of wet cellulose (50 g of dry powder, 110 ml of water) in plastic 0.5 l boxes with a diameter of 10 cm, insuring sufficient aeration. Under these conditions the worms may be kept for weeks, provided the cellulose is refreshed every 2–3 days.

The soil consisted of a mixture of sandy garden soil, clay, peat and refuse of cocoa-beans, ground and extracted for fats and theobromine and enriched with lime. The worms had been kept in a comparable soil for months before the treatment in cellulose as described.

The soil was repeatedly sieved and mixed and afterwards quickly weighed into bags of thin polyethylene-film (0.05 mm) which is hardly permeable to water, but very much so to CO_2 and moderately to O_2; the bags were sealed at once. The 'flat size' of the bags was 11 cm, the length 24 cm, the contents 200 g of soil.

Because of the difficulty of thoroughly mixing truly wet soil, the moisture content was purposely kept too low for the experiment. After filling the bags, random samples

FIG. 2. Free-hanging bags of thin polyethylene-film, used in determining feed uptake of earthworms.

were taken, the moisture content of which was determined to check on the uniformity of the material. An accurately measured amount of water was added to the remaining bags to attain a moisture content which had been found to be near optimal for that particular soil.

The bags were sealed again and hung freely in a room at 8 °C for at least a week, to allow the explosive microbial activity to subside as well as the subsequent CO_2-production always following the mixing of soil.

To facilitate hanging the bags, a double seam was sealed at the top, in which a strip of non-sticking Dymo-tape was enclosed with the necessary code figures and a hole for hanging (Fig. 2).

In the experiment, every hour 8 specimens of *L. terrestris* in batches of 4 were put into bags with soil; the bags were sealed and hung free in a room at 8 °C. After two hours the worms were taken out, quickly rinsed in tap water and put individually in petri dishes on wet, ash-free filter paper discs. After 1, 3 and 5 days they were transferred to clean petri dishes with filter paper. After 6 or 7 days, the worms were weighed and the filters plus excrements were dried and ignited. The rest after ignition was taken as a measure for the feed uptake.

Discussion

The method used in determining O_2-uptake has the advantage of being fully independent of atmospheric pressure, because the apparatus is air-tight and no corrections are needed.

To prevent the worms from drying out during the experiment, apart from the wet nylon cloth on which they were lying, the concentration of the NaOH-solution was kept low, *viz.* 0.05 N = 0.1 osmolar. This is lower than the osmotic value of the body fluids of the worms, which roughly is 0.15 osmolar.

In the respiration experiment 72 specimens of *L. terrestris* were used with a fresh weight of 177.8 g and a total dry weight of 26.1 g or 14.7% of dry matter. Every figure is the average of 363 g·hrs.

The shape of the graph for oxygen uptake (Fig. 3) roughly resembles that found by Ralph (1957), the maximum and minimum falling however earlier. Cole (1957) rightly argues that in this type of study, the time of maxima and minima is partly influenced by the time at wich the experiment is started. Moreover Ralph in his studies used sliding averages which also may influence the peaks in his graphs.

The difference between maximum and minimum O_2-uptake in the present study is very large, the maximum being 4 times the minimum, whereas in Ralph's work this factor is only 1.3.

The level of O_2-consumption is extremely high compared to data from Johnson (1942), who only found 38.7–45.2 µl of O_2-/g·hr, his maximum being slightly lower than our minimum, or from Raffy (1930) finding 25–105 µl/g·hr.

The graph in Fig. 3, apart from a definite tendency to a greater O_2-consumption during the period from 0–12 hrs than from 12–24 hrs, also shows a number of secondary maxima and minima. These are thought to be real, but they should have been camouflaged if only sliding averages had been given.

One of the difficulties in determining feed uptake is that the disturbance, brought

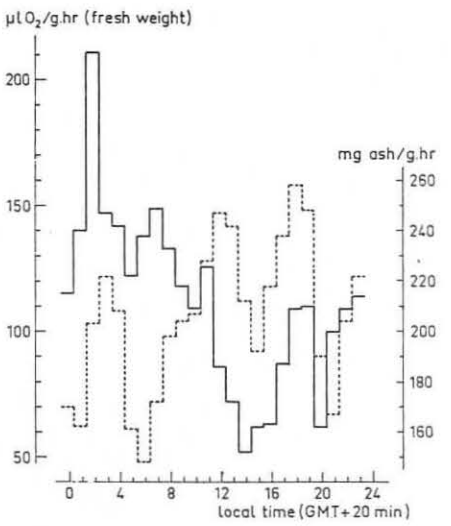

FIG. 3. ——— O_2-uptake by *L. terrestris* in respiration chamber (*cf.* Fig. 1); – – – – – – feed uptake by *L. terrestris* in plastic bags (*cf.* Fig. 2).

about by putting the worms into the soil, causes them to crawl around for some time. This time-lag leads to the time factor being less reliable. Therefore, it is not advisable to take periods of feed uptake shorter than 2 hours. In reality this period will be something between 115 and 90 minutes, but it cannot be exactly determined.

An attempt was made to form two groups of worms which were put into soil at the same time, one remaining in the soil for 2 hours, the other for 3 hours. The feed uptake in 2 hours was subtracted from that in 3 hours. It was found, however, that an unpractically large number of animals was required to determine the feed uptake of 1 hour in this way.

For the graph on feed uptake in Fig. 3 two graphs were constructed, one showing the feed uptake for the periods 0–2, 2–4, etc., the other for 1–3, 3–5, etc. The two graphs fitted nicely; the one presented is the graphical average of the two and therefore actually a sliding average.

Contrary to the oxygen uptake, feed uptake is lower in the period from 0–12 hrs than from 12–24 hrs, but the same secondary maxima and minima are present. They are, however, 1 hr retarded. This shift of the maxima and minima may partly be put down to the time-lag before feed uptake starts, but part of it seems to be real.

It is not within the scope of this article to theorize on the meaning of this phenomenon, but undoubtedly many interesting problems are raised for further studies.

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